2015 West Virginia Mosquito Surveillance Plan

I. Introduction

The West Virginia Mosquito Surveillance Program is responsible for providing timely data on mosquito populations in the state. Mosquito data is primarily collected from May to October when mosquitoes are most active. Laboratory testing of mosquitoes enhances mosquito surveillance by identifying areas where infected mosquitoes were located. This data can also be used to guide control operations and to evaluate control methods.

Four mosquito-borne diseases (all arboviruses) are the focus of the program's surveillance efforts: La Crosse encephalitis (LAC), West Nile encephalitis (WN), eastern equine encephalitis (EEE), and St. Louis encephalitis (SLE). LAC is the predominant mosquito-borne disease in West Virginia and has the highest incidence in the south-central part of the state. In 2012, WN experienced a reemergence nationally and West Virginia reported its highest number of human cases to date (n=9). Cases of WN have been distributed throughout the state. Although no human or equine cases of eastern equine encephalitis have occurred in West Virginia, this virus has been found in birds from West Virginia and horses in neighboring states. Cases of SLE have not been identified in West Virginia since the 1970s. Monitoring for new, invasive mosquito species and species that could transmit novel mosquito-borne diseases is another function of the WV Mosquito Surveillance program.

For arboviral surveillance purposes, CDC recommends that mosquito surveillance systems be tailored according to the probability of arbovirus activity and the resources available for surveillance (CDC, 2013). This plan outlines the activities that will be used by the program to conduct mosquito surveillance during 2015.

II. Objectives

Following are the primary objectives of the West Virginia Mosquito Surveillance program:

1. Identify and describe the mosquito vector composition in West Virginia including:
    a. Vector composition at human case sites
    b. The distribution of exotic mosquito vectors (ex. Asian bush mosquito [*Aedes japonicus]* and Asian tiger mosquito [*Aedes albopictus*])
    c. Mosquito species of public health concern found in neighboring states but unreported in West Virginia (ex. *Culiseta melanura*, *Culex tarsalis*)
2. Monitor for the introduction of invasive mosquito species not established in West Virginia (ex. *Aedes aegypti*);
3. Identify mosquito species that are the cause of citizen complaints and determine whether they are important vector species;
4. Identify mosquito breeding habitats for larval control purposes;
5. Define the geographic area affected by mosquitoes originating from identified habitats and the geographic area that needs to be treated for adult mosquito control;
6. Determine state level mosquito infection rates for arboviruses in different vector species;
7. Provide data to counties participating in mosquito surveillance with regard to:
    a. Local mosquito infection rates for arboviruses
    b. Pertinent mosquito control measures based on geographic location and

developmental stage of mosquitoes;

c. Develop action threshold for mosquito-borne disease

8. Determine the effectiveness of mosquito control measures;
9. Determining the seasonal activity patterns of local mosquito species.

III. Surveillance Plan

Mosquito surveillance in West Virginia involves the following activities: collecting and identifying mosquitoes, testing mosquitoes for certain arboviruses at the WV Office of Laboratory Services (OLS), and providing data feedback to public health partners. Collecting and identifying mosquitoes will involve four primary activities: responding to citizen complaints, conducting site visits for human cases, conducting adult mosquito surveillance, and conducting larval mosquito surveillance. Details for each of these activities are outlined in this section.

Citizen Complaints

Citizen complaints about adult mosquito activity or about potential breeding habitats are useful in mosquito surveillance, especially when establishing a new surveillance program in an area where the mosquito breeding habitats and/or areas within adult mosquito flight range have not yet been identified. Maintaining records of citizen complaints can contribute toward identification and mapping of mosquito problem areas.

State Health Responsibilities

1. Record detailed information from mosquito complaint calls. These calls can be taken by the “Epi on-call” or the entomologist. If the call is from a citizen, the information will be provided to the local health department.
2. Prioritize complaints for further investigation based on call information (what time the mosquitoes were active, whether they were biting, what their biting behavior was, how large the mosquitoes were, what the mosquitoes looked like, whether there are any suspected breeding grounds nearby, etc.).
3. For calls determined as needing follow up, an investigation will be completed through visitation and direct observation and mosquito trapping and testing (where applicable).
4. Report recommendations and/or findings from an investigation back to the caller and local health department.

Local Health Responsibilities

1. Report citizen mosquito complaints to DIDE.
2. As able, assist the entomologist in follow up and investigation of mosquito complaints.
3. Follow recommendations from the entomologist regarding public education and/or mosquito control activities. If assistance is needed, please consult with DIDE staff.

Environmental Assessment of Mosquito-Borne Disease Case Sites

Environmental assessments are conducted at the home sites of cases to examine environmental factors that could have played a role in disease transmission and to provide prevention information to citizens, including mosquito pest management measures.

State Health Responsibilities

1. Maintain an up-to-date arboviral case investigation form that can be used for the environmental assessment of human arbovirus cases.

2. As able, the entomologist will accompany local health department staff during the environmental assessments of human arbovirus cases.
3. Provide guidance for environmental assessments of human cases of novel or emerging mosquito-borne diseases (ex. dengue fever, chikungunya).
4. Maintain and provide education material to local health departments that can be given to citizens during environmental assessments.

Local Health Responsibilities

1. Conduct an environmental assessment of human arboviral cases and report the findings to DIDE using the WVEDSS case report form.
2. Provide prevention education about arboviruses to citizens during environmental assessments, including specific recommendations for controlling mosquitoes around the home. If possible, mosquito abatement activities should be completed including application of biological pesticides (ex. *Bacillus thuringiensis* var. *israelensis* [*Bti])* or removal of standing water and/or water-holding containers.
3. Become familiar with DIDE's Integrated Pest Management plan and employ these guidelines when possible.

Mosquito Surveillance

The distribution of mosquito-borne diseases is based upon the spatial range of their mosquito vectors. Mosquito surveillance can help characterize the population of endemic mosquito species and monitor for new and exotic species. Mosquito surveillance involves the application of different strategies and practices because different mosquito species and developmental stages have their own unique biology. Appropriate species (see list of vectors of adult mosquitoes in Appendix 1) will be collected, pooled and submitted to the West Virginia Office of Laboratory Services (OLS) for arboviral testing. This section outlines the plan for collecting adult mosquitoes and larval specimens, testing adult mosquitoes at OLS, and calculating mosquito infection rates.

Adult Surveillance

Many models of mosquito-borne disease transmission relate human disease incidence to adult mosquito activity (ex. mosquito population density, number of bites per day) as this is the only developmental stage capable of transmitting infections to humans and the immature stages are susceptible to high mortality. Multiple surveillance techniques, such as traps and aspirator collections, have been devised to collect adult mosquitoes and to monitor their activity. Trapping is widely used, but day-to-day success may be variable due to changes in environmental conditions (i.e. wind, air temperature, and rainfall). Therefore, it is often advisable to use several types of traps at a single trap site to collect a representative sample of the species active at that location. Gravid and light traps are two common traps used to collect adult mosquitoes and will be the primary traps used during 2015.

A. Gravid trap (**Fig. 1**): The gravid trap is designed to collect gravid mosquitoes and is among the most important mosquito traps used for surveillance. Gravid mosquitoes are mosquitoes that are carrying eggs and are seeking a site to lay them. In West Virginia, the gravid trap will be used to capture vectors of WN (*Culex pipiens*, *Culex restuans*) and LAC (*Aedes japonicus*). Good places to set these traps include areas surrounding sewage lagoons and sewage treatment facilities (including small package plants and large municipal systems).

B. Light trap (**Fig. 2**): Light traps are one of the standard tools for arboviral surveillance. The advantages to the light trap are that it is portable, easily powered by a 6-volt lantern battery, attracts a relatively wide variety of species (particularly nocturnal and floodwater and marsh mosquitoes) and is the best trap to use for identifying and monitoring the species in a specific locality. The disadvantages to the light trap include that it may only be marginally or poorly attractive to mosquitoes caught by gravid traps and it should be used with an attractant, like CO_2, for maximum effectiveness. In addition, the infection rate calculated from mosquitoes collected in light traps may be comparatively low as the females may not have had a blood meal and become infected with a pathogen.

During 2015, light traps will be used to capture vectors of WN (*Culex pipiens, Culex restuans*), LAC (*Aedes albopictus*, *Aedes triseriatus*), EEE (*Coquilletidia perturbans*), dengue fever (*Aedes albopictus*), chikungunya (*Aedes albopictus*), and malaria (*Anopheles quadrimaculatus*, *Anopheles punctipennis*, *Anopheles barberi*).

Three gravid traps and three light traps will be used to collect adult mosquitoes at each collecting locality. At this time, the WV Mosquito Surveillance program does not plan to routinely use mechanical aspiration, oviposition traps or BG Sentinel traps for mosquito surveillance. See Appendix 3 for additional information on these trap types.

State Health Responsibilities

1. Conduct active adult mosquito surveillance using gravid traps and light traps from May through October. Locations of traps will be determined by the public health entomologist in consultation with local health departments and other interested parties.
2. Record the following information for each collecting event: collecting locality, date of sampling, collection method, species, species abundance, and mosquito testing results. This information will be retained in an electronic database.
3. Hire summer interns to assist with mosquito surveillance by May 2015.
4. Provide training and equipment to counties wishing to conduct adult mosquito trapping in their jurisdiction.
5. Encourage counties around the state to conduct mosquito trapping to better describe mosquito distribution.

Local Health Responsibilities

1. Provide assistance to entomologist, if able, when adult mosquito trapping is being conducting in jurisdiction.
2. Contact entomologist if wanting to participate in adult mosquito trapping.

Larval Surveillance (Modified from O'Malley, 1989)

Whereas adult surveillance provides information about mosquito infection rates and mosquito biting activity, larval surveillance provides information regarding sources of mosquito populations. Larvae sampling procedures require less time, disturb less space, and are less intrusive than passive adult mosquito traps. Based upon larval surveillance, larvae control measures may be initiated to reduce future adult mosquito-related problems.

Larval surveillance will be the surveillance method of choice for environmental assessments of case sites and community outreach in areas with a high incidence of LAC cases.

State Health Responsibilities

1. As able, conduct larval sampling with local health department staff during environmental assessments of case sites.
2. Process larval samples at OLS.
3. Report larval surveillance results back to the local health department for additional follow up with cases, as needed.
4. Provide guidance and equipment to local health department staff on collecting larval samples and appropriate prevention measures.

Local Health Responsibilities

1. Attempt to notify the entomologist when performing an environmental assessment at a case site.
2. Provide larval samples to the entomologist from case sites, as able. Follow processing and preservation guidelines outlined in this protocol (see Appendix 2).
3. Provide appropriate prevention measures to cases and the public based on larval surveillance data.

OLS Mosquito Submission and Testing

Viral testing of adult mosquitoes provides data for calculation of mosquito infection rates. Currently, OLS can test mosquitoes for WN, LAC, EEE, and SLE. Following are the recommended steps in submitting specimens to OLS.

1. For local health departments performing adult mosquito surveillance in their jurisdiction, mosquitoes should be frozen as soon as they are collected from the field. Once frozen mosquitoes, can be submitted in the trap bag or the trap bag can be emptied and the mosquitoes submitted in a cryovial.

2. Mosquito specimens can be sent to OLS through the mail, dropped off at OLS, or by working with the public health entomologist to pick up specimens. Specimens submitted by mail should shipped to:

   Eric Dotseth
   Mosquito Identification
   Office of Laboratory Services
   167 11th Avenue
   South Charleston, WV 25303

3. The collecting locality, collection date, and type of trap used to capture the mosquitoes should be sent with mosquito submission.

4. Once received by the public health entomologist (Eric Dotseth 304-356-4020; Eric.J.Dotseth@wv.gov), he will complete an OLS mosquito pool submission form and assign the sample a mosquito pool number before submitting it for arboviral testing. It is a good idea to inform the public health entomologist or the OLS microbiology staff that a mosquito sample is being sent. Contact information for OLS staff responsible for arboviral testing of mosquitoes is provided below:

   a. OLS staff
      i. Christi Clark - 304-558-3530 ext. 2606; Christi.D.Clark@wv.gov
      ii. Lindsay Barr - 304-558-3530 ext. 2403; Lindsay.R.Barr@wv.gov

5. Mosquitoes will be tested using real-time reverse transcription polymerase chain reaction (PCR). This method allows a targeted sequence of viral RNA to be transcribed into cDNA which is then amplified millions of times in just a few hours. Real-time reverse transcription PCR is useful for detecting very low amounts of RNA molecules. Mechanical homogenization of mosquitoes will begin with two copper beads in each pool and will be lysed in guanidine isothyiocyanate-containing RNA lysis buffer. Qiagen® QIAamp RNeasy Mini kit will be used to isolate viral RNA from mosquito tissue. AgPath-ID one-step RT-PCR with detection enhancer will be used for PCR amplification. Polymerase chain reactions will be run using the ABI 7500FAST. Biosearch Technologies provides the primers and Taqman probes. The Centers for Disease and Control provide controls for validation. For further details about arboviral screening protocol, contact Christi Clark at OLS.

6. The Office of Laboratory Services will disseminate results of arboviral testing to cooperating local health departments and the public health entomologist. The public health entomologist will deliver the results to the CDC. Results of the survey will be maintained electronically by the public health entomologist and OLS.

State Health Responsibilities

1. Identify collected mosquitoes to species. Due to difficulty in differentiating field damaged specimens, *Culex pipiens* and *Culex restuans* will be designated as *Culex pipiens*/*restuans*.
2. Pool mosquitoes into sets of 10 to 50 individual, adult mosquitoes of the same genus from the same location and same collection week. Special projects may require the arboviral testing of mosquitoes by species (ex. the Asian bush mosquito *Aedes japonicus*).
3. Certain important vector species may be submitted in pools of as few as 10 mosquitoes.
4. Submit mosquito pools to OLS for testing using the OLS Mosquito Pool Submission Form.
5. Report mosquito testing results to CDC via ArboNET.

Office of Laboratory Services Responsibilities

1. Test mosquitoes based on genus using RT-PCR. Under special circumstances, arboviral testing may be conducted based upon species (ex. the Asian bush mosquito *Aedes japonicus*). Mosquitoes will be tested for LAC, EEE, WN, and SLE.
2. Report mosquito test results to the public health entomologist, the zoonotic disease data analyst, and the local health department in the county where the mosquitoes were collected.
3. Maintain mosquito test results electronically.

Local Health Responsibilities

1. For local health departments conducting mosquito surveillance in their own jurisdiction, submit mosquitoes specimens based on the guidelines outlined above.
2. Review mosquito test results sent by OLS for mosquitoes tested in their jurisdiction. This information can be used to provide education to the public or guide mosquito control activities.

3. If a lab result is received for a positive mosquito pool in the LHD jurisdiction, refer to prevention actions listed under moderate WN activity (see section V below). Consult with the public health entomologist regarding specific larval and adult mosquito prevention activities for the site where the positive mosquito pool was located.
4. During May through October, monitor local mosquito test results and statewide MIR activity in determining the level of prevention activities that should be implemented.

Calculating Mosquito Infection Rates

There are many ways to discuss infection information collected through arboviral testing of mosquito pools. For example, isolation of virus in a mosquito pool demonstrates infection in the mosquito species collected from a locality. Although valuable as an indicator of arboviral infection in the locality sampled, this method does not ascertain the prevalence of infection in the mosquito population. Discussing arboviral infection as either a function of the number of infected mosquito pools or the percentage of infected mosquito pools relates infection incidence to mosquito population size; however, the number of mosquito pools and number of mosquito specimens per pool must remain constant for any comparative value.

Infection rates provide the most accurate definition of infection prevalence in the mosquito population. Mosquito infection rate is a better indicator of virus incidence in the vector population because this value relates incidence of infection with a population indicator. Mosquito infection rates do not require the numbers of mosquito pools or the mosquito pool size to remain constant. The minimum infection rate (MIR):

$$MIR = \frac{virus\ positive\ mosquito\ pools}{total\ number\ of\ mosquitoes\ tested} \times 1{,}000$$

Due to its simplicity and historical value amongst many mosquito surveillance programs, MIR will be the measure of infection used by the West Virginia Mosquito Surveillance Program.

The level of arboviral infection in the mosquito population will determine the course of mosquito-borne disease management. Before 2013, LAC virus was isolated from very few LAC competent vectors in West Virginia. The addition of detection enhancer during PCR amplification cycles in 2013 resulted in increased LAC virus detection in mosquitoes. A statewide MIR > 5 per 1000 *Culex* spp. (a primary WN vector) mosquitoes for two consecutive weeks may be an indicator of increased human WN activity in the following weeks. It is difficult to quantify and relate infection rates among other mosquito species to future WN activity in humans in West Virginia. In some years of low human WN incidence, the WN MIR was greater than 5 per 1000 *Culex* spp; however, these high infection rates did not continue through the following week. Examining data across two or more weeks will accommodate for fluctuations caused by low mosquito yield (attributable to local weather or trap mishaps). Early seasonal peaks in WN virus infection in *Culex* spp. could be exaggerated when low numbers of mosquitoes are collected. Since MIR is the ratio of the number of positive mosquito pools to the total number of mosquitoes tested, a small number of infected mosquitoes could result in a high calculated infection rate. Therefore, it is recommended to view infection rates during the early season (when the *Culex* species populations are starting to rise) with caution when the weekly *Culex* spp. count is less than 200 and infection is limited to a single *Culex* spp. pool.

State Health Responsibilities

1. Use mosquito test results to calculate the minimum infection rate (MIR) for each collection week.

2. Provide MIR data to local health departments through the “West Virginia Vectorborne Disease Surveillance Report”.
3. Use weekly MIR data to monitor trends in mosquito viral activity and determine if additional prevention measures need to be implemented.

Local Health Responsibilities
1. Review MIR data when sent by DIDE.
2. Implement recommended control measures based on MIR data. See “Recommendations for Responding to Increased Mosquito Viral Activity” in section V.

IV. Surveillance Feedback

Surveillance feedback is an important component to any surveillance system and provides information to assist with implementing appropriate control measures. ArboNET provides national arbovirus surveillance data for states and counties through their website (http://www.cdc.gov/ncidod/dvbid/westnile/USGS_frame.html)

State Health Responsibilities
1. Report mosquito testing results to CDC through ArboNET. Mosquito data provided to AboNET includes county of collection, collecting date (MMWR week), taxonomic identification (ex. genus, species), number of mosquitoes in the mosquito pool, and arbovirus isolated from the mosquito pool. Information about mosquito pools without arboviral infection is also provided.
2. During May through October, provide regular surveillance updates to public health partners via dissemination of the “West Virginia Vectorborne Disease Surveillance Report”. Depending on vector and disease activity this report may be sent weekly or bi-weekly. This report summarizes human and non-human cases of all vectorborne diseases. Reports will be emailed and posted to the DIDE website.
3. Analyze mosquito data on a yearly basis and summarize this data in the Annual Mosquitoborne Disease Report. This report will be emailed and posted to the DIDE website.

Local Health Responsibilities
1. Review the ‘West Virginia Vectorborne Disease Surveillance Report’ for current information on mosquito activity throughout arbovirus season.
2. Forward the weekly/biweekly “West Virginia Vectorborne Disease Surveillance Report” to public health partners in their jurisdiction.
3. Review and share the annual mosquito-borne disease report when released each year.

V. Prevention

Prevention activities as they relate to mosquito surveillance can be divided into two categories: Integrated Pest Management and response to elevated mosquito viral activity based on MIRs.

Integrated Pest Management

Integrated Pest Management (IPM) programs are a sustainable approach to managing mosquitoes by combining biological, cultural, physical, and chemical tools in a way that

minimizes economic, health, and environmental costs. IPM programs use current, comprehensive information on mosquito species biology and their specific interactions with the environment. This information, in combination with available pest control methods, is used to manage mosquito populations by the most economical means and with the least possible hazard to people, property, and the environment. See the IPM document provided by DIDE at: http://www.dhhr.wv.gov/oeps/disease/Zoonosis/Mosquito/Pages/IPM.aspx

The following activities can be used as part of an IPM approach to control mosquito populations in West Virginia:

- Remove/modify mosquito breeding habitats by emptying and removing any water-holding containers, filling tree holes with thermal insulation, and performing land modification that allows for proper drainage.
- Participate in community tire drives that remove old tires from an area. The West Virginia Department of Environmental Protection Rehabilitation Environmental Action Plan (REAP) was created to rid the state of unsightly litter and provides collection events where West Virginia citizens can dispose of tires without the cost of disposal. Additional information about REAP, PPOD, and local tire collecting events is available on their website (http://www.dep.wv.gov/dlr/reap/tires/Pages/default.aspx).
- Use biological pesticides for managing mosquito larvae. *Bacillus thuringiensis israelensis* (*Bti)* and *Bacillus sphaericus* (*Bs*) are available commercially as 'biorational' larvicides. Since their active ingredient is only triggered when ingested by target insects, *Bti* and *Bs* are safe to handle and a special pesticide applicator license is not required for application.

Note on pesticide use: Pesticides can be useful in managing mosquito adults as they can immediately reduce adult mosquito populations below the acceptable injury level. However, pesticide application is only recommended in certain situations (ex. near high human density gatherings in the event vector MIR approaches the injury level) and is considered the least effective control method as it does not provide long-term residual control and can have negative environmental impacts. The Environmental Protection Agency (EPA) encourages non-chemical mosquito control measures. Although many pesticides for mosquito control are available in West Virginia, some pesticides are labeled as 'restricted use.' To be able to apply a 'restricted use' pesticide, the applicator must have a license demonstrating knowledge about pesticide application and safety. Information about pesticides registered in West Virginia is available at http://www.kellysolutions.com/WV. Certification for restricted use pesticide application is completed through the West Virginia Department of Agriculture. The following websites have more information about pesticide license application:
http://www.wvagriculture.org/Division_Webpages/READ-regulatory.htm
http://www.pestnetwork.com/continue/new%20state/westvirginia1.html

Recommendations for Responding to Increased Mosquito Viral Activity

The level of human risk can be categorized based on mosquito viral activity as calculated by MIRs. The following section outlines various risk levels based on mosquito WN activity and provides appropriate prevention actions to take for each level. Actions implemented in previous levels should be maintained through the subsequent level (ex. responses at the 'low human risk level' should continue through the 'medium human risk level'.)

Level 1: Low human risk for WN

CONDITIONS

- No WN infection in mosquito pools (often seen during late spring and early summer)

ACTION

- Routine public education (general symptoms of WN, general mosquito management, personal protection against mosquitoes)
- Routine trainings to local health departments about mosquito-borne disease epidemiology, surveillance results from previous year, and case ascertainment and investigation
- Routine press release notices (general symptoms of WN, general mosquito management, personal protection against mosquitoes)
- Routine notifications to physicians and veterinarians (upcoming mosquito season, preferred diagnostic test methods, promoting OLS services)
- Routine reminder to labs to send human samples to OLS
- Routine memo to local health department about resources available through West Virginia Department of Health & Human Resources
- Routine mosquito and arbovirus surveillance activities
- Continue to notify public about arbovirus surveillance results
- Maintain mosquito larval control
- Inventory pesticides and pesticide control equipment

Level 2: Moderate human risk for WN

CONDITIONS

- WN infection in some mosquito pools in the county but statewide MIR < 5 / 1000 *Culex* spp. or statewide MIR > 5 / 1000 *Culex* spp. for only one week

ACTION

- Enhance public health education message (signs and symptoms of encephalitis, encourage patients to seek medical care if required, inform the public about pesticide applications)
- Ensure notification of key agencies of WN activity including local offices of emergency services
- Conduct case site investigations associated with human or equine cases
- Increase adult mosquito surveillance in surveyed areas
- Increase number of mosquito pools tested for WN
- Increase surveillance and control of mosquito larvae
- Conduct localized, limited chemical control of adult mosquitoes based upon consultation with state public health entomologist
- Contact commercial applicators in anticipation of large scale adulticiding

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Level 3: High human risk for WN

CONDITIONS

- Statewide MIR > 5 / 1000 *Culex* spp. for two continuous weeks.

ACTION

- Conduct full scale media campaign (general symptoms of WN, encourage patients to seek medical care if required, general mosquito management practices, personal protection against mosquitoes, and mosquito-borne disease surveillance activities)

- Alert physicians, veterinarians, and local health departments about the impending severity through Health Alert Network
- Enhance information to healthcare providers
- Conduct active human case investigation
- Continue case site investigations of human and equine cases
- Increase geographic coverage of adult mosquito surveillance
- Continue enhanced larval surveillance and control of mosquito larvae
- Initiate more extensive adult mosquito control
- Consider localized aerial adulticide application in rural environments

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Conditions for each risk level may change based upon other surveillance indicators. The relationship between some surveillance indicators (ex. human cases, equine cases, WN virus positive dead birds) and human risk is not completely understood for West Virginia. For example, a county with a WN human case but no infected mosquito pools (possibly because the county does not have an active mosquito surveillance program) would probably be considered at medium risk for WN. Or having multiple WN human cases and infected mosquito pools in a county but a statewide MIR < 5 / 1000 *Culex* spp. mosquitoes would warrant the county as being a being at high risk for WN. For questions about appropriate preventive actions based on WN activity, please consult with DIDE.

VI. Resources

Centers for Disease Control and Prevention
http://www.cdc.gov/ncidod/dvbid/westnile/education.htm.

ArboNET, the national arbovirus surveillance system
http://www.cdc.gov/ncidod/dvbid/westnile/USGS_frame.html

Division of Infectious Disease Epidemiology
www.dide.wv.gov

VII. References

Centers for Disease Control and Prevention. 2013 West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control,4th revision
http://www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf

Darsie, R. F. & R. A. Ward. 2005. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. Gainesville, Florida: University Press of Florida.

Dohm, D. J., M. L. O'Guinn, & M. J. Turell. 2002. Effect of environmental temperature on the ability of *Culex pipiens* (Diptera: Culicidae) to transmit West Nile virus. *Journal of Medical Entomology* **39** (**1**): 221-225.

Gujral, I. B., E. C. Zielinski-Gutierrez, A. LeBailly, & R. Nasci. 2007. Behavioral risks for West Nile virus disease, northern Colorado. *Emerging Infectious Diseases* **13** (**3**): 419-425.

McNelly, J. R., 1989. The CDC Trap as a Special Monitoring Tool. Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc. 1989, pp 26-33. http://www-rci.rutgers.edu/~insects/cdctrap.htm

O'Malley, C, M., 1989. Guidelines for Larval Surveillance. Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc. 1989, pp 45-55.http://www-rci.rutgers.edu/%7Einsects/larvsurv.htm

Appendix 1 – Mosquito Species Associated with Arbovirus Transmission Cycle

All adult mosquitoes collected during the surveillance season will be tested for WN, EEE, LAC, and SLE. Listed below are the mosquito species which have been found carrying these pathogens. This information was derived from laboratory or field studies published in peer-reviewed publications or ArboNET, the national arbovirus surveillance system (http://www.cdc.gov/westnile/resources/pdfs/Mosquito%20Species%201999-2012.pdf). Only mosquito species found in or near West Virginia have been listed.

WN
Aedes albopictus
Aedes atropalpus
Aedes canadensis
Aedes dorsalis
Aedes cinereus
Aedes japonicus
Aedes sollicitans
Aedes sticticus
Aedes tormentor
Aedes triseriatus
Aedes trivittatus
Aedes vexans
Anopheles barberi
Anopheles crucians
Anopheles punctipennis
Anopheles quadrimaculatus
Coquillettidia perturbans
Culex erraticus
Culex pipiens
Culex restuans
Culex salinarius
Culex territans
Culiseta inornata
Orthopodoymia signifera
Psorophora ciliata
Psorophora columbiae
Psorophora ferox
Psorophora howardii
Uranotaenia sapphirina

EEE
Aedes canadensis
Aedes cinereus
Aedes japonicus
Aedes sollicitans
Aedes triseriatus
Aedes trivittatus
Aedes vexans
Anopheles punctipennis
Anopheles quadrimaculatus
Culex erraticus
Culex restuans
Culex salinarius
Culiseta melanura
Coquillettidia perturbans
Psorophora ferox
Uranotaenia sapphirina

LAC
Aedes albopictus
Aedes atropalpus
Aedes japonicus
Aedes sollicitans
Aedes triseriatus
Aedes trivittatus
Aedes vexans
Anopheles punctipennis
Coquillettidia perturbans
Culex pipiens
Culex restuans

SLE
Aedes atropalpus
Aedes dorsalis
Aedes japonicus
Aedes vexans
Culex erraticus
Culex pipiens
Culex restuans
Culex salinarius

Appendix 2 - Guidance for Local Health Departments Conducting Adult Mosquito Surveillance

Although the mosquito surveillance is maintained by the state public health entomologist, the West Virginia Office of Laboratory Services (OLS), and seasonal, temporary state employees, other local agencies and volunteers are encouraged to participate. Local health departments can provide valuable assistance by collecting mosquitoes in their respective jurisdictions. Local mosquito data can be used by local health departments to direct their mosquito-control activities. The state public health entomologist can provide collecting equipment and technical expertise to local health agencies and volunteers. See the guidance below for additional information.

I. Trap Site Selection
   a. Mosquito Habitat
      1. For WN mosquito vectors (*Culex spp*.), look for areas with stagnant, shallow pools of water with rich, organic matter.
      2. For LAC mosquito vectors (eastern treehole mosquito [*Aedes triseriatus]*), look for areas with water-holding trees and containers.
   b. Accessibility of Site
      1. Mosquito surveyors must be able to physically access the site within the confines of an average work day. Evaluate the site for physical hazards such as rugged terrain.
      2. Permission must be acquired from the property owner. Dialogue between investigators, property owners, and local health departments will insure compliance and alleviate fears from concerned citizens.
      3. Consider if the site will be accessible for future mosquito surveillance in following years.
   c. Any additional projects beyond mosquito surveillance will also influence the selection of mosquito surveillance sites. Sewage septic systems have been built based upon the discovery of WNV in the local breeding mosquito population. Cleanup projects have been initiated following the discovery of LAC mosquito larvae in the tires. Scientific studies can be derived from state mosquito monitoring data. For example, the impact of two invasive LAC competent, mosquito species (*Aedes albopictus*, *Aedes japonicus*) on LAC epidemiology is not entirely understood.

II. Mosquito Traps and Collection Times
   a. Gravid traps and light traps are the primary traps used in West Virginia.
   b. To accommodate for mosquito development time and mosquito surveillance schedules, sites should be sampled once a week.
   c. Traps and samples should be collected 24 hours after operation at a surveillance site in order to reduce mosquito damage, mosquito decomposition, arbovirus decomposition, and battery damage.

d. Mosquito collecting procedures should be maintained at a site throughout the arboviral surveillance season to collect information about mosquito and human pathogen seasonal activity.

e. Mosquito collecting procedures should also remain consistent between sites in order to understand mosquito species density and arboviral infection rate across the state. Surveillance sites can be used each year to allow a historical record of mosquito activity.

III. Data and Mosquito Identification

a. The following data should be collected and sent with each shipment.

1. Collector's name
2. Collecting locality (state, county, city/town/township, address, geographic coordinates)
3. Collecting dates
4. Sampling method (ex. gravid trap, light trap)

b. Volunteers do not need to identify mosquitoes to species. However, listed below are the appropriate procedures for individuals who personally wish to identify the mosquitoes to species

1. Empty the trap bag and place the label from the trap bag into a white plastic tray for sorting or into a container for transporting and sorting later.
2. Species identifications should be performed with a dissecting microscope.
3. Sort the mosquitoes into piles of similarly looking kinds. Other organisms caught in the trap may be discarded.
4. The mosquitoes can then be identified using the appropriate taxonomic key and placed in containers. "**Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico**" will identify the mosquitoes species most recently active in West Virginia.
5. All identifications will be confirmed by the public health entomologist.
6. Once identified, the data should be recorded on a standardized field form and copies forwarded to the public health entomologist at OLS along with the mosquitoes collected. Ensure that all specimen containers have been labeled with **site name/number, collection date, county, collector, and species**.

IV. Preservation and Submission of Mosquitoes for Testing at OLS

All specimens, whether larval or adult, live or dead, should be treated in a manner to limit damage to the specimens. Avoid jarring, grinding, excessive handling of specimens. In addition, avoid direct sunlight and extremes in cold and heat.

Adult Mosquitoes

1. Carefully handle the trap bag from the gravid and light traps so as not to destroy the mosquitoes.

2. Do not submit specimens alive. The mosquitoes, after being trapped, usually die quickly. A short time in a field vehicle is enough to ensure the demise of the mosquitoes on a warm summer day. However, a better method is to leave the trap bag with the mosquitoes in a freezer.
3. After the mosquitoes have died, the trap bag or another secure container can be used to send the specimens to OLS.
4. Ensure all specimen containers are labeled with site name/number, collection date, county, and collector.
5. Send specimens to:

    Eric Dotseth
    Mosquito Identification
    Office of Laboratory Services
    167 11th Avenue
    South Charleston, WV 25303

Mosquito species may be retained for archival purposes by the public health entomologist. Archived specimens serve as a physical record of a species occupying a given area at a specific time. Specimens may also be retained for future taxonomic study or instruction in mosquito identification.

Larvae

Larval mosquitoes can be processed in one of two ways. The mosquitoes can be returned to the lab, put in rearing chambers, and then identified as adults, or they can be processed and preserved in the field for later identification in the laboratory as larvae.

a. **Laboratory rearing**: Larval mosquitoes and the water (up to ½ gallon) from the larval habitat should be placed in a plastic bag (1 gallon zip-lock) or water-holding container with lid. The bag or container should be labeled with appropriate collection information (**site name/number, collection date, county, collector, habitat type, number of dips**). The plastic bag or container can then be placed in a cooler for transportation to the lab. No ice is necessary, but the specimens should **not** be allowed to overheat or freeze in a vehicle. The mosquitoes and the water from the sample should be placed into a rearing chamber. If there is too much water, remove some using a turkey baster. A small amount of tropical fish food should be finely ground and put into the water with the larvae (only a pinch is needed). The rearing chamber should then be labeled with the appropriate collection information **(site name/number, collection date, county, collector, habitat type, number of dips**). The rearing chambers should be placed out of direct sunlight, however they should get ambient lighting from outside in order to receive the appropriate cues for emerging as adults. Once adults have emerged, they should be aspirated from the rearing chamber, and placed in the freezer. Freeze the adult mosquito long enough to ensure the mosquito dies so that it can identified under the microscope. Once identified, the data and mosquitoes should be processed as indicated in the identification section of adult mosquitoes.
b. **Non-reared larvae**: Mosquitoes to be preserved in the field or laboratory can be removed from the sample water and placed into a small container/vial containing 70 percent ethanol or rubbing alcohol for preservation. Mosquito larvae must be in their

fourth instar (stage) to be properly identified. The specimens then can be identified when needed. All specimens must be processed as described in the identification section for adult mosquitoes.

Appendix 3 – Mosquito Trap Information

Traps for Adult Mosquito Surveillance

Gravid Traps (Figure 1)

Gravid traps use a small electric fan, typically powered by a 6-volt lantern battery, to suck up the mosquitoes that visit the bait container and blow them into a collection bag. Gravid traps are baited with a tub of odorous infusion (tea or fermented brew) made from water and organic material (e.g. grass clippings, hay, dead leaves, yeast, pelleted rabbit chow, horse or chicken manure, etc.).

Fig. 1. Components and set-up of a gravid trap

When trapping *Culex* species it is best to use a bait infusion made from a recommended formula. Gravid *Culex* mosquitoes are attracted to the smelly water infusion as a place to lay their eggs which is similar to the stagnant, high organic content water brew these mosquitoes normally breed in. There is a higher probability of collecting virus-infected mosquitoes in a gravid trap than in a light trap because gravid traps attract female mosquitoes that have already taken at least one blood meal and are ready to lay eggs. The

species collected may vary by where the trap is set and/or what formula is used to make the infusion bait. Traps are best set under bushes, under porches, in tall grass, or out of the wind in areas close to where target vector species may be seeking a place to lay eggs. General locations with some shade provided by a tree canopy or other source is desirable When trapping any mosquito species, gravid traps are best set sometime between 2:00 and 4:00 PM and collected the next day around 8:00 or 9:00 AM. To reduce viral decomposition and damage to the mosquitoes, gravid traps should only be run for a single day. Gravid traps collect live mosquitoes, which are to be used for identification and testing.

Formula for gravid trap bait infusion for *Culex* mosquitoes

Bait for gravid traps used in mosquito surveillance can be obtained through the public health entomologist or by obtaining the ingredients and following the directions below:

Ingredients

1. Large plastic trashcan (40-50 gallons) with a tight fitting lid.
2. 30 gallons of water
3. 1 pound of straw or hay
4. 5 grams of brewers yeast
5. 5 grams of lactalbumin powder

Mixing Directions

1. Place the trashcan in a place where it will get direct sunlight for several hours per day
2. Fill the can with 30 gallons of water
3. Stir 1 pound of straw into the 30 gallons of water
4. Add 5 grams of brewers yeast
5. Add 5 grams of lactalbumin
6. Stir the mixture
7. Place lid on trashcan and let the mixture brew for five days, stirring at least once each day

Bait Usage Directions

After a period of about five days the bait will be ready to use. **Note:** This particular brew will have a foul odor (somewhat similar to that of sewage), but will be highly attractive to *Culex pipiens* and *Culex restuans*. If you use this bait and do not catch either of the above mentioned *Culex* species, there probably were not any active in that area where the gravid trap was set. Be careful not to leave the lid off the trashcan because the odor of this bait may offend neighbors, and may attract swarms of egg laying *Culex pipiens* every night.

It is convenient to pour the finished bait into a 2.5 gallon, wide-mouth, container to carry it to your trap sites (an empty 2.5 gallon, plastic cat litter container works well for this purpose). After use, the bait can either be dumped, or it can be poured back into the carrying container for repeated use. If the bait is to *be* reused repeatedly, add several granules of Altosid^{TM} larvicide to the bait to prevent the development of mosquitoes from eggs that have been laid in it.

When this particular bait is freshly made, it is **not** attractive to *Ae. albopictus* or container-breeding *Aedes* species. However, after about three weeks of usage, this bait becomes slightly less attractive to *Cx. pipiens* or *Cx. restuans*, and becomes more attractive to the *Ae. albopictus* and container-breeding *Aedes* species. If collection of *Cx. pipiens* and *Cx. restuans* is your primary goal, you should start a fresh batch of bait every month. You can keep the older bait and use it when trapping specifically for the container breeding *Aedes* species.

Other bait mixtures include but are not limited to:

1. Large plastic trashcan (40-50 gallons) with a tight fitting lid.
2. 30 gallons of water
3. 1 pound of straw or hay
4. 1 pound of grass clippings
5. 1 Tablespoon of brewers yeast
6. 3 lbs. dried chicken manure (available at garden centers)

Follow mixing and usage directions for the above formula. To save time in mixing bait the straw can be spread on the lawn prior to a mowing. After mowing rake the chopped straw and grass mixture and freeze in two pound blocks.

Light traps (Figure 2)

For special surveillance of short duration, light trap is an efficient, reliable surveillance tool. This trap can be used to assess a citizen's complaint, check the success of an adulticide or gather arbovirus information. The light trap is portable, battery powered, and efficient.

The light trap uses a small light source to attract and capture mosquitoes that are seeking hosts for a blood meal. Different light wavelengths can attract different mosquito species. And, since light traps use only a small light source, these traps attract relatively few non-mosquito insect species, such as beetles and moths, that can damage the trapped mosquitoes. Unfortunately, using only a light will make this trap ineffective due to competing light sources (ex. full moon). However, baiting the trap with CO_2 increases both the number of mosquitoes and range of species collected as compared to traps using light as the sole attractant. Use of CO_2 to bait the trap requires a supply of dry ice, or canisters of compressed CO_2. A trap baited with CO_2 may require 2-3 pounds of dry ice or compressed gas per night. Light traps may also be baited with octenol and/or a human skin non-toxic chemical lure, which are designed to attract blood-seeking females. The chemical lure should be attached to the outside of the tube containing the fan assemblage with a rubber band. The combination of the chemicals and CO_2 may make the trap more attractive to some mosquitoes.

Fig. 2. Light trap

Guidelines for CDC Light Traps: The following guidelines are offered to minimize variability in the use of CDC light traps for mosquito surveillance. (Modified from McNally, 1989)

1. Whenever possible, use the CDC trap with a dry ice supplement. A quantity of 2.5 to 3.0 lbs of pelletized or block dry ice in an insulated container (2 quart cooler) will mimic a large mammal's respiration and last long enough to cover the usual mid-afternoon to dawn trapping period.

2. If the capture of excessive non-mosquito insect species is a problem, or vandalism or theft of the trap a concern, remove the light source when dry ice is used as an attractant; the absence of light will eliminate other photopositive

insects from the collection, increasing the efficiency of identification. It will also make the trap less visible to vandals and thieves.

3. Hang the dry ice directly above, or adjacent to, and slightly below, the aluminum lid of the CDC trap to draw mosquitoes as close as possible to the collection fan. Or, if supplied, place the dry ice into the container of the trap or attach the CO_2 hose, depending on what make of trap you are using.

4. Trap at least one hour prior to dusk until one hour after dawn to insure that surveillance is conducted during the primary host-seeking periods for most species. Setting traps earlier in the afternoon will result in the capture of day-biting species. This is especially important when collecting container breeding *Aedes* species . These would be the most important species in communities where La Crosse encephalitis has been a public health problem.

5. To prevent viral decomposition and mosquito damage, traps should only be left running for a single day.

6. Hang the trap so its light is 5-6 ft from ground level unless specific information is needed on canopy dwellers. For most species, this height will provide a reliable indication of activity. For *Ae. albopictus* the lid of the trap should be at waist height when the trap is hanging.

7. Try to set the traps along the edges of habitats to increase trapping efficiency. A trap located strictly in one ecosystem/habitat may exclude certain species; trapping along the edge of a swamp, for example, will provide a picture of those species found not only in the swamp, but also in the nearby upland.

8. Consider **two traps** as the minimum number per site in most situations and compare your data to detect differences that may have been due to outside influences.

9. Be aware that differences do exist in the host seeking behavior of some species and that alterations from these general guidelines may be necessary to get complete surveillance data (**record all trap settings and deviations on the** mosquito surveillance field form). Strictly daylight feeding species will not be accurately represented in dusk-dawn collections. A species that seeks hosts in tree canopies will not be accurately sampled by a trap that is suspended 5 ft from the ground. Whenever possible, become familiar with the host seeking habits of the mosquitoes being surveyed.

Note: Trap should have contact information and warning related to dry ice attached when placed in the field.

BG Sentinel traps (Figure 3)

The BG Sentinel trap attracts and captures mosquitoes using visual cues, olfactory stimuli, and air convection currents associated with vertebrate hosts. The trap is a collapsible container with white gauze covering the opening. In the middle of the white gauze cover is a black catch pipe with an electric fan at the base. Air is drawn through the pipe by this electric fan and mosquitoes caught in the air current are captured in the collection bag. Air exits through the white gauze and generates an ascending air current, similar to convection currents generated by human hosts. Lures containing chemicals generated by living vertebrate hosts are available. Chemical attractants include octenol and BG-Lure (ammonia, lactic acid, and fatty acids found on human skin). Carbon dioxide will act synergistically with these chemical lures to attract more blood-seeking mosquitoes. The black and white color scheme of the BG Sentinel trap has proven useful in other successful mosquito sampling traps. And the low ground design is conducive to the lower strata feeding habitats of certain mosquito species, such as the Asian tiger mosquito (*Ae. albopictus*).

Fig. 3. BG Sentinel trap

Aspirators (Figure 4)

Like a vacuum cleaner, the aspirator uses air suction to collect small insects into a collection vial. Power aspirators range in size from small hand-held battery powered units to larger battery or gasoline powered backpack units. Aspirators are quick, active, and non-obtrusive, and can be used to capture adult mosquitoes which elude gravid and light traps.

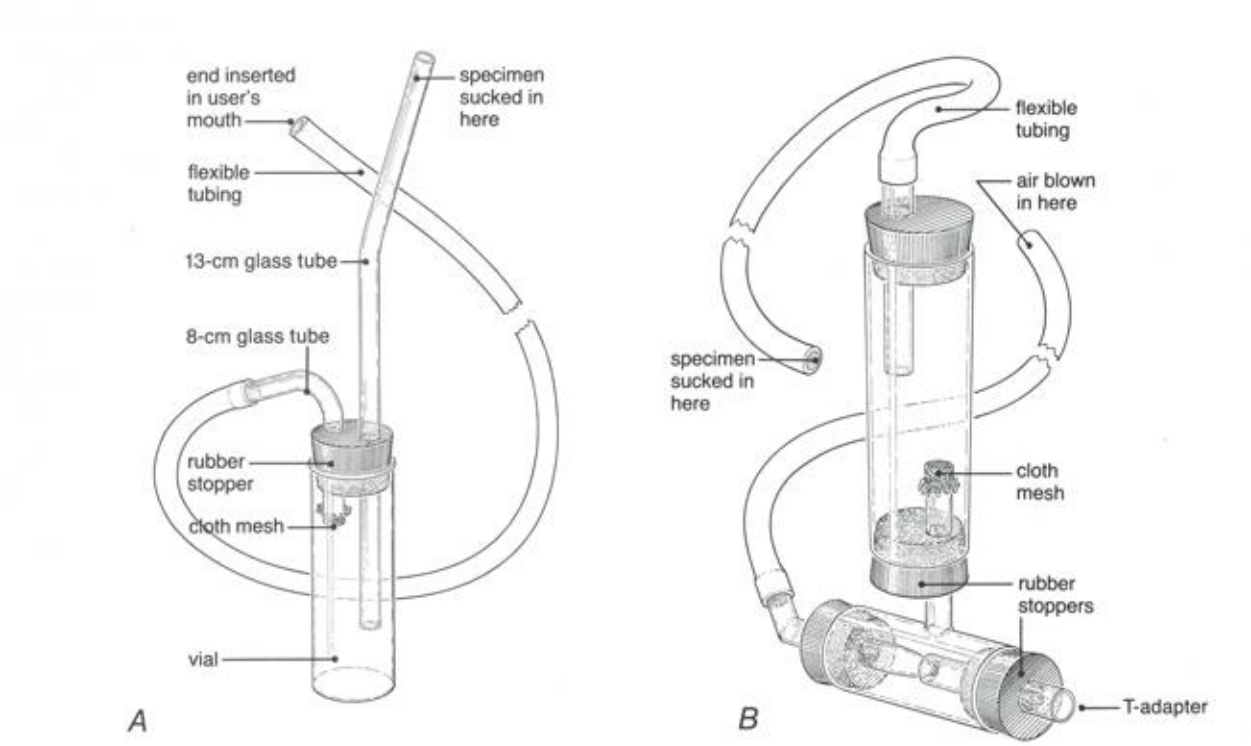

Fig. 4. Aspirator components

Larval Surveillance

Like fly larvae, mosquito larvae lack legs and wings. In addition, mosquito larvae have a cylindrical body with a developed head and no knobs or protuberances from the thorax. Learn to recognize different microhabitats within an area; each one of these should be sampled in order to obtain a comprehensive picture of the area's species composition.

Basic tools

1. Standard, white 400 ml-capacity dipper (**Fig. 5**)
2. An eyedropper; turkey baster, tea strainer, white enamel or plastic pan
3. Boots
4. Vials, plastic bags or some other form of container for collecting larvae including labels for the collections, sharpies for labeling bags, preservative,
5. Mosquito surveillance field form
6. Pencil

7. GPS receiver - used to obtain data for GIS

Fig. 5. Mosquito dipper with turkey baster

C. **Collection Methods:** When searching for mosquito larvae, it is necessary to proceed slowly and carefully. Approach the area to be inspected with caution, as heavy footfalls will create vibrations that disturb larvae and cause them to dive to the bottom. Likewise, avoid disturbance of the water, as this will have the same result. Approach the area to be sampled with the sun in one's face; this prevents shadows that also disturb larvae and cause them to dive. If wind is of significant magnitude sampling should be done on the windward side of the habitat where larvae and pupae will be most heavily concentrated.

The kind of mosquito one is looking for, as well as the type of habitat one is working in, will determine the technique used. If field personnel are familiar with the general breeding habits of the major species found within their county, they will be able to choose the most appropriate technique to obtain the most reliable results. **Dipping** is one of the most effective methods for sampling mosquito larvae. The following seven techniques for sampling mosquito larvae and pupae with the standard pint dipper are effective:

1. <u>The Shallow Skim</u> - *Anopheles* larvae are normally found at the surface of the water among aquatic vegetation or floating debris. *Anopheles* larvae do not have siphons. They can be collected with a shallow, skimming stroke along the surface, with one side of the dipper pressed just below the surface. End the stroke just before the dipper is filled, to prevent overflowing.

2. <u>Partial submersion</u> - Around emergent vegetation, logs and tree stumps, larvae may be drawn into the dipper by submerging one edge so that the water flows rapidly into the dipper. In this method, the dipper is stationary within the water.

3. Complete submersion - Certain Culicine larvae (such as species of *Aedes* and *Psorophora*) are very active and usually dive below the surface when disturbed. In this case, a quick plunge of the dipper below the surface of the water is required, bringing the dipper back up through the submerged larvae. Bring the dipper back up carefully, to avoid losing the larvae with overflow current.

4. Dipper as a background - This is an especially useful technique in woodland pools, for early season species. Submerge the dipper completely within the woodland pool, going down into the bottom litter if necessary. Use the white dipper as a background against which larvae and pupae can be spotted. Come up underneath the larvae with the dipper. Once again, bring the dipper up carefully, to avoid losing its contents.

5. Flow-in method - This method is useful in situations where the water is shallow, with mud, leaf litter, or other debris on the substrate. Specimens can be collected by pushing the dipper down into the material on the bottom and letting the shallow surface water and mosquito larvae flow directly into the dipper.

6. Scraping - This method is used in permanent or semi-permanent habitats containing clumps of vegetation, such as reeds or tussocks. Dip from the water in, towards the tussock, and end by using the dipper to scrape up against the base of the vegetation to dislodge any larvae present.

7. Simple scoop - This is the technique which seems to be most commonly used by field personnel for larval surveillance and is the one referred to in much of the literature as "the standard dipping procedure." The technique involves simply scooping a dipper full of water out of a habitat. It is useful in a wide variety of habitats, especially for collecting *Culex*.

Note: Several species of mosquito are difficult to collect by dipper because their aquatic habitats often occur in containers or other depressions that are too small to sample with a dipper. These include:

- *Ae. albopictus* - tires
- *Aedes atropalpus* - rock pools, tires
- *Ae. japonicus* – rock pools, treeholes, tires, containers

- *Ae.triseriatus* - treeholes, tires, containers
- *Anopheles barberi* - treeholes, tires, containers
- *Coquilletiddia perturbans* - permanent water with emergent vegetation
- *Culiseta melanura* - Cedar and red maple swamps, occasionally tires
- *Orthopodomyia signifera*: - treeholes, tires, containers
- *Toxorhynchites rutilus septentrionalis* - treeholes, tires, containers
- *Wyeomyia smithii* - pitcher plants

The turkey baster is an inexpensive, readily available tool that is very useful for sampling tires, containers and tree-holes. A small white plastic soup ladle will also work well. The tea strainer can be used to concentrate and sort samples. Material collected can then be emptied into a white enamel pan (if preservation or concentrating samples in the field), from which the mosquito larvae are then removed, or poured into a plastic bag or water-holding container if being returned live to the laboratory.

Note: It is important to recognize that whenever dipping for immature mosquitoes, regardless of the technique used, it is important to look for actual presence of larvae before dipping, and to proceed carefully and pay attention to what you are doing.

D. Oviposition trap (**Figure 6**):
Collections of mosquito eggs in oviposition traps are used to detect and monitor container-breeding mosquitoes such as *Ae. triseriatus*, *Ae. japonicus*, *Ae. albopictus*, and the yellow fever mosquito *Aedes aegypti* (not currently found in West Virginia). The oviposition trap can be easily made out of food cans (3 lb. coffee cans), pint jars or 16 oz. aluminum cans painted black inside and outside. The traps are placed in shaded areas at a height no greater than 1.2 m (around 3 ft.) above the ground and filled with water. An oviposition substrate (seed germination paper, muslin, formica, balsa wood, wooden tongue depressor) is then placed vertically inside the container with the water covering about half of the substrate. Gravid females use this substrate to lay eggs just above the water level. Traps are checked every 10 to 14 days to maintain water levels or prevent them from becoming breeding sources. If larvae are found in the trap, then the water should be removed and the trap reset. The ovipositional substrate is periodically collected and returned to the laboratory in a plastic bag. Samples are kept cool and moist during transportation, taking care to avoid too much moisture. Excessive moisture will cause the eggs to begin hatching. Eggs or emergent larvae are then identified and tested for pathogens. At this time, oviposition traps are not used in West Virginia for mosquito surveillance. This is for informational purposes only.

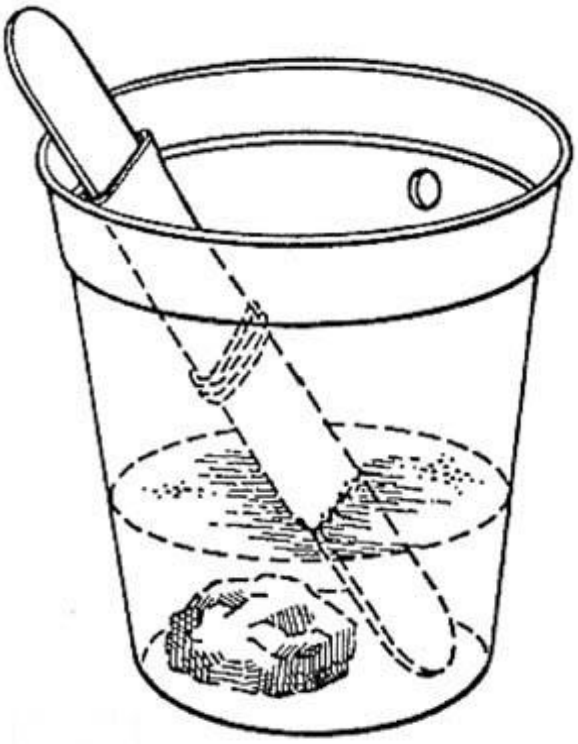

Fig. 6: Oviposition trap